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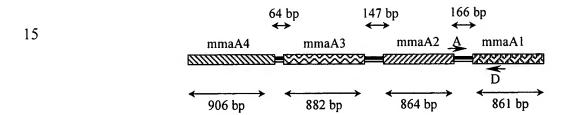


Figure 1: Schematic diagram of methoxy mycolic acid synthase mmaA 4-mmaA 1 gene cluster of mycobacteria and location of forward A, and reverse D primers.

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| ICAACCATTTCAATCGTCGGAGGTTGGCCCCCCGGGAAGATTTCGGTCGCGATGAACTTGAGAAAGCGGGCCAGCC CCTAGCTTGCCCAGCCCAGGTCGATCTTGGCGATCTGGGCCTCTTCCAGCGTCATGTCCTCGCGTTCGAAATGCGC ICTITIGCTGCGTTCATAGTGATTCCGGCTGAGCGTGAGGCCGATGACATTGACGTCGTACTTCTCCACGGCCCGAAC ATGTACCGCTCGTAGACCTCTTCGGACTGGATCGCGATGGCCTCGCTTTTGTGTTCCTGCAGCGCCTCGGCCCACAG GTCGAGGGTCCTGGCGTAATGCGGCTGCAGCGACTGGCGGCGAGTCAGCGTGAAACCCGTCTTCGCCGACTGTTCC ICTTCGGACTGTACGGCGATGGCGCGTTCGCGGGCAGCCTGTAGGTTGGCGGCCCATGCATC<u>GAGAGTCCGTGCGTA</u> GATCGAGGAACAACGCGAAGAAGTCATCCGAAATGTCGTAAGCCGACTGTGACTCTTCGTAATATGGTCTCAGCTT gcatgctcgaggcatgctatccgatacagggccgcactaaaccgcgatcgaatttgcccaggtcagggaacggatatgagcggacgacgagCTACTTGGTCATGGTGAA CTGGGCGACGTTGATTAGGCCTCTGCGGAAGCGCTCCGCGATCCGGTCAGATAGTGCATGAAGTTGTTGTAGACC CGTAGGAGCGTTCGAAGAACGTCAGATACCGCTCCTTTTTGAACGCGTCGAATGCCTCAAAGCTGACGATCCGGTC GAGCGCCCCGCCCCCCCCACCCACGTCGAGTAGCGTCATCCCCGGTTCGAGGTTCAGCTTGTCCAACGCCAGA CATCGGGCGGCAGGATTTTGTGGGCCCGGGCGAAGAAGTCGGCGTGACGATCGTGGCCGAAGTGCTCGAACGCGC 2GATCGACACGATGCGGTCGACGGGCTCGTTGAACTGCTCCCATCCCGCCAGCAACACTCGCCTGTCGCGCGGGGT <u> GTG</u>CTGCTGCAGCAGCTGGACATGCTCGATGGTGAAGCCCGCGGCCTGCGCATTGTCGACAATGTCGGGCTCCGAT CTACTTCGCCAGCGTGAACTGGTTGACGTCGATGTAGCCGACCCGGAACAGCTTGGCGCAGCCGGTCAGGTATTTC ACAACGTGAGCGGCAAGCCGTGGTCGACCATCTGCTGCTGGTCAGGCCGGTGATCGTGTGCAGCAGCACACGC STCCATCTCGTCGAACGACTTCTGCACATGGGCGGCCTGGTTCTTCGACAATGTCAGGCCGACGACGTTGACGTCA GCAGCTGTAGGTCTGGGTCCGGATCCAGGAACAGCCGGAAGAAGAAGTCGTCGGACAGGTCGTAGTGTGCCTGCACGTC CTCGAAGTGCGGCGTTAGGTCGTTGACCATgaggtgtaatgcctttccggaccctaggtggcctttcggtgcttgcacggaacgcaccgatgcttcccctcccc GGCAGCTCGCCCGGGGAAGATCGACTCCCGCAGGAATTTGAGGAATCGAAGGTCGCTCATCGTCAGCGCAATG CCCTGTTCGTGCAGCCACCTGCGGTCGTAGGTGAACAGGCTGTGCAGTAGCATCCGCCCGTCATCGGGCAGGATGT GACGTTCTTCAAACTCTTCCCAGCCCTGCAGCCGGGCCTCGGCGCGCGTTGCGTTCCGATTGCGGCCAGGCGG TCCACCTTGGCCAGTTGCGCCTCTTCCAGCGTCATATCGTCACGCTCGAAATAGGCGCAGGTGTAGACCCAGGTGG IACTGCGCGATCGCGCGCCGCATGGTGGCGCCCCAGCCGCAACCGATATCGAGCAGCGTCATGCCGGGCTGCAGA

Fig. 2: Sequence of mmaA2 and mmaA1 gene with an intergenic region of 166 base pair (shown in lower case. Location of forward A, sequence ID 1 and reverse primer D, sequence ID 2. Both primer sequence is underlined and italisized.

1 2 3 4 5 6 7 8 9 10 11 12 13 14



Fig. 3 PCR amplification of different mycobacterial genomic DNAs with primers A and D (lanes 1- 15): 1. M.avium 2. M.bovis 3. M.chelonae 4. M.fortuitum 5. M.intracellulare 6. M.kansassi 7. M.phlei 8. 100 bp DNA ladder 9. M.marinum 10. M.scrofulaceum 11.M.smegmatis 12. M.szulgai, 13. M.tuberculosis and 14. negative control. AD indicates 363 bp-amplified product.

Spacer 166 bp mmaA1 175 bp

MspI 33 bp
HaeI 47 bp

MspI 244 bp
HaeI 219 bp

Fig. 4: line diagram showing restriction endonuclease map of HaeI and MspI within AD.

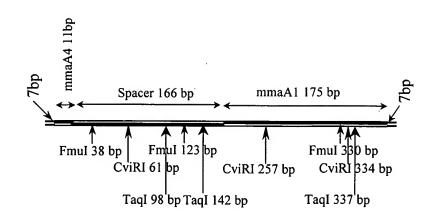


Fig. 5: line diagram showing restriction endonuclease map of FmuI, CviRI and TaqI within AD.



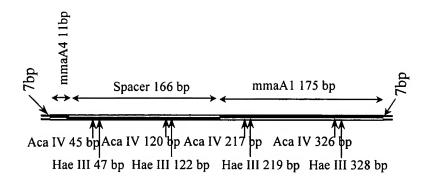


Fig. 6: Restriction map of AD showing distribution of the sites of restriction endonucleases AcaIV and HaeIII.

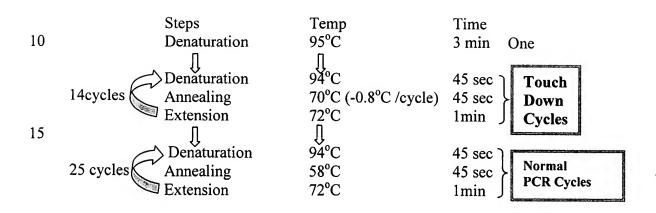


Fig. 7: Line diagram showing different steps of PCR reaction